REMARKS

Claims 1, 2, 4-9, and 11-18 are pending after entry of this paper. Claims 1-4, 9-15, and 18 have been rejected. Claims 5-8, 16 and 17 have been withdrawn and claims 3 and 10 have been cancelled without prejudice. Applicants reserve the right to pursue withdrawn and cancelled claims in a divisional or continuing application.

Claim 1 has been amended to replace the phrase "a buffer ability similar as that with concentration . . . " with the phrase "a buffer capacity equivalent to the concentration . . . ." Support may be found throughout the instant application and claims.

Claims 1, 11 and 14 have been amended to add the term "nonionic" to a step of treating with a surfactant in the claimed method. Support may be found throughout the instant application and claims.

Finally, claim 13 has been amended to correct a misspelling of the term "enterolysin."

No new matter has been introduced by this response. Reconsideration and withdrawal of the pending rejections are respectfully requested.

Information Disclosure Statement

The Patent Office confirmed at page 2 of the Office Action that the information disclosure statement (IDS) submitted on May 18, 2010 has been considered as made evident by a provided copy of the PTO-1449 form (Information Disclosure Citation). However, applicants respectfully note that two citations (WO 95/05461 and EP 0552571) in the above-identified form were crossed out, *i.e.*, not considered, although upon applicants' substantial review, it is not readily apparent to the reasons why these references were crossed out. While the Patent Office

mentions that the citations that are not in English will not be considered unless provided with an English translation or with an English abstract, these two references do not qualify since both were in English and did not require a separate translation. Hence, applicants respectfully ask the Patent Office to reconsider their position and admit these two citations.

Response to Rejections under 35 U.S.C. §112, second paragraph

Claims 1, 2, 4, 9, 11-15, and 17 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite. Specifically, the Patent Office contends that it is unclear what constitutes a buffer with "similar" ability. (Office Action; pg. 3). Applicants respectfully disagree.

However, in order to expedite prosecution and without disclaimer of, or prejudice to, the subject matter recited therein, applicants have amended claim 1 to replace the phrase "a buffer ability similar as that with concentration . . . " with the phrase "a buffer capacity equivalent to the concentration . . . ." Thereby, those skilled in the art would be readily apprised of the meets and bounds of the pending claims. In particular, applicants wish to draw the Examiner's attention to the definition of "buffer capacity" provided in the Exhibit A,

> Buffer capacity is a measure of this ability to resist pH change and depends on both the absolute and relative component concentrations. In absolute terms, the more concentrated the components of a buffer, the greater the buffer capacity. . . . It's important to realize that the pH of a buffer is distinct from its buffer capacity. A buffer made of equal volumes of 1.0 M CH3COOH and 1.0 M CH3COO- has the same pH (4.74) as a buffer made of equal volumes of 0.10 M CH3COOH and 0.10 M CH3COO-, but the more concentrated buffer has a much larger capacity for resisting a pH change.
>
> Buffer capacity is also affected by the relative concentrations of the buffer components. As a buffer functions, the concentration of one component increases relative to the other. Because the ratio of

> these concentrations determines the pH, the less the ratio changes, the less the pH changes. For a given addition of acid or base, the buffer-component concentration ratio changes less when the concentrations are similar than when they are different.

Therefore, those skilled in the art would be fully apprised of what constitutes a medium with an equivalent buffer capacity, i.e., the amount of H_3O^+ or ^-OH added would be about the same to obtain the same pH change. Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §112, second paragraph, rejection of claims 1, 2, 4, 9, 11-15, and 17.

Claims 13 has been rejected under 35 U.S.C. §112, second paragraph as being indefinite because according to the Patent Office, the term "Enterolysine" can not be definitively ascertained.

In order to expedite prosecution and without disclaimer of, or prejudice to, the subject matter recited therein, applicants have amended claim 13 to replace the term "Enterolysine" with the term "Enterolysin." Support for this amendment may be found, for example, in paragraph [0010] of the specification "it was shown that bacteria could also be completely lysed by using a bacteriocin having lytic activity such as Enterolysin[[e]] instead of Achromopeptidase." Enterolysin is a bacteriocin having lytic activity. (see Exhibit B; *Applied and Environmental Microbiology*, May 2003, p. 2975 – 2984). Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §112, second paragraph, rejection of claims 13.

Response to Rejections under 35 U.S.C. §103

Claims 1, 2, 9, 11, 12 and 18 have been rejected under 35 U.S.C. §103(a) as being unpatentable over Hsih et al. (*J Food Prot.* 2001 Nov; 64(11):1744-50) in view of Kearney et al. (U.S. 5,589,335), and further in view of Brasher et al. (*Curr Microbiol.* 1998 Aug; 37(2):101-7). Specifically, the Patent Office contends that since UP broth of Hsih appears to be analogous to the medium No. 17 used by Applicants on pg. 23 of specification (0.5 g glucose), the reference reads on the claimed method. Nonetheless, the Patent Office acknowledges that Hsih does not teach treating bacterial samples with a lytic enzyme, a surfactant, and a protein denaturant. To compensate for the alleged shortcomings of Hsih, the Patent Office looks to Kearney because Kerney allegedly teaches lysing a bacterial sample comprising *E. coli* and *L. monocytogenes* with lysozyme, bacteriocin, and proteinase K. Finally, the Patent Office acknowledges that the combination of Hsih and Kearney still fails to teach the use of a surfactant during cellular lysis in claim 1. To compensate for the alleged shortcomings of Hsih and Kearney, the Patent Office contends that it would be obvious based on Brasher because it allegedly teaches lysing a bacterial sample comprising *E. coli* and *S. typhimurium* with SDS and proteinase K followed by centrifugation and DNA precipitation with alcohol. (Office Action; pg. 5-6). Applicants respectfully disagree with the arguments set forth in the Office Action with respect to the cited references and the attained conclusion.

First, applicants do not dispute that Hsih teaches a method of detecting *Salmonella spp.* and *Listeria monocytogenes* in food samples, but the cited reference must be examined as a whole (see MPEP 2141.02). In particular, Hsih teaches a method of detecting *Salmonella spp.* and *Listeria monocytogenes* in food samples by combining the immunomagnetic separation (IMS) technique and the multiplex polymerase chain reaction (PCR) method (see

Abstract; emphasis added). Hsih describes that *Salmonella spp.* grow more rapidly than *L. monocytogenes* in UP broth, and in situations when both of these target organisms were present together during the growth step in UP broth, false-negative or ambiguous results were found for *L. monocytogenes* (Hsih, page 1748, left column, lines 8 to 12). To solve these problems, Hsih proposes the use of the immunomagnetic separation (IMS) step prior to PCR (Hsih, page 1748, left column, line 24 to right column, line 2) and describes immunomagnetic beads that were selected specific for *Listeria spp.* and *Salmonella spp.* On the other hand, the instant invention does not require the immunomagnetic separation (IMS) step in order to provide high sensitivity in multiple microorganism detection. Thus, those skilled in the art would have to substantially modify how the method of Hsih attaints its results in order to arrive at the claimed invention. As stipulated in MPEP 2143.01, the modifications suggested by the Patent Office cannot render the prior art reference unsatisfactory for its intended purpose. At least on this ground, those skilled in the art would not look to Hsih in order to arrive at the claimed method.

Second, to compensate for the alleged shortcomings of Hsih, the Patent Office looks to Kearney because Kerney allegedly teaches lysing a bacterial sample comprising *E. coli* and *L. monocytogenes* with lysozyme, bacteriocin, and proteinase K. However, applicants respectfully wish to point out that Kearney relates to novel organic salts and combination-salts, having unique and useful properties related to the hybridization and detection of nucleic acids (column 4, lines 60 to 63). In particular, Kearney describes the use of various mixtures of reagents that denature nucleic acids such as a mixture of tetramethyl-ammonium and trichloroacetate, a mixture of tetramethylammonium and trifluoroacetate and others, with a mixture of guanidium thiocyanate (GuSCN) with tetramethylammonium and trifluoroacetate being the most preferred (column 6, lines 48 to 52).

Regarding the protocol for hybridization and elution/recapture cited by the Patent Office, applicants respectfully assert that the cited reference must be considered as a whole, i.e., in its entirety, including portions that would lead away from the claimed invention (MPEP 2141.02). Kearney describes that the addition of Proteinase K to a final concentration of 2.5 mg/ml in the reagent results in there being no digestion by RNase, and being at least 50% more intact rRNA than without the addition, and at least 50% of 5 mg/ml of BSA is solubilized (column 5, lines 40 to 44). On the other hand, in the present invention where microorganisms are detected by amplifying chromosomal DNA by PCR, a skilled artisan could not conceive to use protein kinase K because, according to Kearney, it has been confirmed to suppress digestion by RNase.

Third, to compensate for the alleged shortcomings of Hsih and Kearney with regard the use of a surfactant during cellular lysis in claim 1, the Patent Office contends that it would be obvious based on Brasher because it allegedly teaches lysing a bacterial sample comprising *E. coli* and *S. typhimurium* with SDS and proteinase K followed by centrifugation and DNA precipitation with alcohol. However, applicants wish to draw the Patent Office's attention to the fact that Brasher teaches the simultaneous detection of *E. coli*, an indicator of fetal contamination and microbial pathogen, *Salmonella typhimurim*, *Vibrio vulnificus*, *V. Cholerae*, and *V, parahaemolyticus*, respectively, without the use of bacteriocin or lytic enzyme. These are all gram-negative bacteria, and it is well known that DNA extraction is much more easier as compared to gram-positive bacteria or the combination of two. For example, *Listeria* grows at a low temperature and proliferates slower compared to *Salmonella spp.* and *E. coli O157*. Thus, the DNA extraction method(s) that work for the species that have a Gram-negative cell wall do not necessarily mean that the same extraction method(s) would work for species that

have a Gram-positive cell wall and vice versa. Therefore, a skilled artisan would not look to Brasher that provides a species-specific teachings to reach the technical idea related to DNA extraction, for detecting a 2 or more particular microorganism having different properties including *Listeria monocytogenes*, being gram-positive bacteria.

Additionally, Brasher describes that DNA was purified with chloroform-isoamyl alcohol followed by centrifugation and that further purification of the DNA in the supernatant was achieved by extracting with phenol-chloroform-isoamyl alcohol. Phenol and chloroform are dangerous and harmful organic solvent that are not suitable for examination of pathogens in food manufacturing sites. (Specification as filed; para. [0009] page 7). On the other hand, it is readily noted in the instant specification that "even phenol or chloroform treatment is not performed, protein being soluble to a level sufficient to be detected by PCR without problem can be removed by alcohol precipitation or according to the added level of DNA extraction solution (2 μl per 50 μl of PCR reaction solution) "(paragraph [0011]). Thus, Brasher and the instant invention are quite different in the effect and the utilization of a Brasher method would make the Hsih method unsatisfactory for its intended purpose of testing *Listeria moncytogenes* in food and/or food manufacturing sites.

In view of the aforementioned remarks and claim amendments, applicants respectfully asset that the instant invention is not made obvious by Hsih in view of Kearney and Brasher. Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claims 1, 2, 9, 11, 12 and 18 as being obvious over Hsih in view of Kearney and Brasher.

Claim 4 has been rejected under 35 U.S.C. §103(a) as being unpatentable over Hsih et al. in view of Kearney et al. and Brasher et al. as applied to claim 1 and in further view of Rimick et al. (U.S. 6,468,743), Buck et al. (Biotechniques, 27(3), 528-536, 1999) and Lowe et al. (Nucleic Acid Research 18(7), 1990, 1757-1761). Specifically, the Patent Office argues that the only shortcoming of Hsih, Kearney and Brasher is that these references are silent about the primer sequences recited in SEQ ID Nos. 5 and 6. (Office Action; pg. 7). In particular, the Patent Office states that "since the claimed sequences simply represent structural homologs of those sequences disclosed in the prior art, and concerning which a biochemist of ordinary skill would attempt to obtain alternate compounds with improved properties, the claimed primers (SEQ ID Nos: 5 and 6) is *prima facie* obvious over the cited references in the absence of secondary considerations." (Office Action; pg. 9). Applicants, however, respectfully disagree with the attained conclusion.

For instance, the Patent Office points to SEQ ID No: 59 disclosed in Table 3 of Rimick, which is one of the primers for detecting *L. monocytogenes*. Although, the primer sequence of SEQ ID No. 5 of the present invention is identical to 20 bp from 5' end of SEQ ID No: 59 of Rimick, it must be also noted that the primer sequence of SEQ ID No: 6 of the present application is identical to the 20 bp from position 21 from 5' end of SEQ ID No: 59 of Rimick. Therefore, a skilled artisan could not conceive a primer pair shown by SEQ ID Nos: 5 and 6 based on SEQ ID No: 59 of Rimick and would simply suggest to those skilled in art to go on a fishing expedition to identify an appropriate primer pair from potentially million possible primer pairs. Hence, the combined teachings of Hsih, Kearney, Brasher, Rimick, Buck, and Lowe cannot be used as the basis for *prima facie* obviousness rejection. Therefore, applicants

respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claim 4 as being obvious over the cited art.

Claim 14 has been rejected under 35 U.S.C. §103(a) as being unpatentable over Hsih et al. in view of Kearney et al. and Brasher et al. as applied to claim 1 and in further view of Bussey et al. (U.S. 6,011,148). Specifically, the Patent Office holds that "it would have been *prima facie* obvious to a person of ordinary skill in the art at the time of invention to utilize Tween 20 in the lysis mixture of Hsih since the prior art highlights Tween 20 as a functional equivalent of SDS." Applicants respectfully disagree.

However, in order to expedite prosecution and without disclaimer of, or prejudice to, the subject matter recited therein, applicants have amended the presently pending claims to clarify that the surfactant is an nonionic surfactant. Support may be found in paragraph [0019] of the specification as filed ("As surfactants, anion surfactant, cation surfactant, amphoteric surfactant, nonionic surfactant can be used. Among these, ethylene oxide condensate of sorbitan monolaurate which is a nonionic surfactant, more specifically Tween 20, are preferably used.").

Moreover, the method of Bussey provides that the chromosomal DNA is precipitated together with protein and cell debris and removed. On the other hand, in the instant method the chromosomal DNA is amplified by PCR, followed by analysis, to detect microorganisms. Thus, considering these are two absolutely different methods, those skilled in the art would not use a nonionic surfactant such as Tween 20 for completely different method described in the instant disclosure. Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claim 14 as being obvious over the above-cited art .

Claim 15 has been rejected under 35 U.S.C. §103(a) as being unpatentable over Hsih et al. in view of Kearney et al. and Brasher et al. as applied to claim 1 and in further view of Aznar et al. (of record). According to the Patent Office, Aznar teaches lysing a bacterial sample with guanidium isothiocyanate (Office Action; pg. 13). In particular, the Patent Office contends that "it would have been *prima facie* obvious to a person skilled in the art at the time of invention to utilize guanidium isothiocyanate in the lysis mixture of Hsih since the prior art highlights guanidium isothiocyanate as a functional equivalent of proteinase K." (Office Action; pg. 13). Applicants respectfully disagree.

As it is stated in the above, the cited combination of Hsih, Kearney and Brasher fails to teach the claimed method and the shortcomings described above would not be compensated by Aznar. Moreover, contrary to the position taken by the Patent Office, applicants respectfully assert that Proteinase K is not an equivalent of guanidium isothiocyanate, and it cannot be said that the invention of claim 15 is obvious, even by combining with Hsih, Kearney, and Brasher with Aznar. In particular, Proteinase K is a lytic enzyme, whereas guanidium isothiocyante is a protein denaturant. Applicants wish to draw the Patent Office's attention to paragraph [0019] of the specification as filed, which states that "[e]xamples of the above lytic enzymes include: Achromopeptidase, lysozyme, proteinase K, chitosanase, chitinase, b-1,3-Glucanase, Zymolyase and Cellulase" and "[a]s for the above protein denaturants, guanidine isothiocyanate, urea, guanidine hydrochloride, trichloroacetate, SDS, Triton X-100 and deoxycholate can be exemplified"

In view of the aforementioned remarks and claim amendments, applicants respectfully asset that the instant invention is not made obvious by Hsih in view of Kearney and

Brasher and in further view of Aznar. Therefore, applicants respectfully request reconsideration and withdrawal of the 35 U.S.C. §103(a) rejection of claim 15 as being obvious over the above-cited art.

Dependent Claims

The applicants have not independently addressed all of the rejections of the dependent claims. The applicants submit that for at least similar reasons as to why independent claim(s) 1 from which all of the dependent claims 2, 4, 9, 11-15 and 18 depend are believed allowable as discussed *supra*, the dependent claims are also allowable. The applicants however, reserve the right to address any individual rejections of the dependent claims and present independent bases for allowance for the dependent claims should such be necessary or appropriate.

CONCLUSION

Based on the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the restriction requirement imposed on the pending claims and allowance of this application. Favorable action by the Examiner is earnestly solicited.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **50-4827**, Order No. 1004451.001US.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **50-4827**, Order No. 1004451.001US.

Respectfully submitted,
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